Structural Investigations on Cyt.b6/f-Complex and PS I-Complex from the Cyanobacterium Synechocystis PCC 6803

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The role of Photosystem I polypeptides involved in the photoreduction of $NADP^+$.

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Photosystem I is a membrane bound multiprotein-pigment complex consisting of a reaction centre,(P700), a primary electron acceptor (A_0), an intermediate redox component (A_1), and three iron-sulphur centres FeS_X, FeS_A and FeS_B. Iron-sulphur centres $FeS_{A/B}$ are bound to a 9kDa polypeptide which may be isolated as a functional protein. An intact core complex consisting of functional P700, A_0, A_1, and FeS_X is isolated by treatment of PSI with urea which removes some of the smaller peripheral polypeptides. Removal of the $FeS_{A/B}$ apoprotein is monitored by measuring the rereduction of $P700^+$ by back reaction from electron acceptors following laser excitation. Intact PSI shows a back reaction half time ($T_{1/2}$) of 10 ms reflecting a rereduction from $FeS_{A/B}$. This $T_{1/2}$ decreases to 1 ms on treatment with 6.8M urea with a full recovery to 10ms on incubation of the core protein with $FeS_{A/B}$ apoprotein. EPR data confirms the restoration of low temperature $FeS_{A/B}$ photoreduction in reconstituted PSI particles. Digitonin extracted PSI reduces $NADP^+$. Urea treatment results in the loss of $NADP^+$ photoreduction, however the reconstituted $FeS_{A/B}$\core particle regains 90% of the original NADP reduction activity. There are a number of polypeptides involved in the reconstitution of $NADP^+$ photoreduction and the function of these will be discussed.

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THE SUBUNIT STOICHIOMETRY OF PHOTOSYSTEM 1 REACTION CENTER.

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Quantitative amino acid analysis using hydrolysis and dabsylation (Aminochrome) of electroblotted PVDF membrane was applied for SDS-PAGE resolved Photosystem 1 reaction center subunits for the determination of stoichiometry. Highly specific and quantitative ELISA technique using antibody raised against each protein was also employed to determine relative amount of Photosystem 1 subunits in terms of P_{700} in a number of reaction center preparations. The 1:1 ratio was obtained for PsaA, PsaB, PsaC, PsaD and PsaE in highly purified preparations from spinach chloroplasts. On the other hand, in crude preparations, a ratio of more than three molecules per P_{700} was observed for PsaD, while other subunits remained the same.

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CHARACTERIZATION OF PSAF GENE PRODUCT

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The PSI complex prepared from cucumber cotyledons, which contains 80 chlorophylls per P700 and eight polypeptides of psaA/psaB (65/63 kDa), psaD (20 kDa), psaE (19.5 kDa), psaF (18.5 kDa), psaH (7.6 kDa), psaC (5.8 kDa), and an unknown gene (17.5 kDa) has been shown to catalyzes the light-dependent transfer of electrons from plastocyanin (PC) to ferredoxin. The psaF gene product was easily depleted from the complex that inactivates the complex at the site of electron transfer from PC to photooxidized P700. PC was specifically cross-linked to the psaF gene product of the PSI complex. Kinetic propeties of the cross-linked adducts were studied. cDNA of psaF gene was isolated from cucumber and sequenced. The interactions between PC and PSI complex were discussed based on its nucleotide sequence.

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STRUCTURAL INVESTIGATIONS ON CYT.B6/F-COMPLEX AND PS I-COMPLEX FROM THE CYANOBACTERIUM *SYNECHOCYSTIS* PCC 6803

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A cytochrome b6/f-complex was isolated from *S.* 6803 and highly purified by HPLC, involving new column-techniques. The apparent molecular mass of this complex was determined for the first time by a combination of analytical HPLC and electron microscopy. Both results agree very well with a monomeric complex, assuming a 1:1 stochiometry of all subunits. From averaged images of the electron micrographs, the shape of the complex and the dimensions of the top- and side-view projections could be determined. The subunit composition (by SDS-PAGE), immuno-blots, spectroscopic data and the redox potentials of the components will be presented.

For the PS I-complex of *S.* 6803, conditions could be found which cause an extreme shift towards either monomeric or trimeric PS I as seen by an HPLC-analysis of the extracted complexes. As no substantial reorganization of solubilized complexes has been observed, this may reflect a possible conversion of both complexes in the native membrane. A model for the physiological significance of an equilibium between both forms will be presented.

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THEORY ON THE WAVELENGTH-DEPENDENT POLARITY OF THE LIGHT-GRADIENT PHOTOVOLTAGE

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The light-gradient photovoltage from photosynthetic organisms and organelles arises from the transmembrane primary charge separation in the reaction centers. The asymmetry of photoinduced dipoles is currently explained by the higher excitation of the membrane side of a vesicle facing the light source than of the opposite side. Together with the known orientation of reaction centers, this explanation predicts unequivocally the polarity of the photovoltage. However, dependent on the wavelength of excitation, a polarity opposite to the one expected has often been observed. Here we report on a theoretical treatment of light propagation and light interference in pigmented and nonpigmented multilayers. A model calculation is carried out demonstrating the wavelength-dependent light distribution as well as the relative photovoltage and its polarity. The model is tested by comparison with new photovoltage data in chloroplasts. Light distribution is found to be more uniform than predicted from pure absorption behaviour, leading to smaller gradients of excitation.

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SYNTHESIS AND ASSEMBLY OF PHOTOSYSTEM I SUBUNITS IN WILDTYPE BARLEY AND THE PHOTOSYSTEM I DEFICIENT BARLEY MUTANT *viridis-zb*^{63}.

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Photosystem I (PS I) of higher plants contains 12 polypeptide subunits, five of which are encoded in the chloroplast genome and seven of which are encoded in the nuclear genome. The PS I complex accumulates in light but is undetectable in etiolated plants. In the present study, the synthesis and turn-over of PS I subunits was analyzed in barley by in vivo and in organello pulse-chase experiments. In etiolated plants and in etioplasts none of the subunits could be detected except for the plastid encoded iron-sulfur protein PSI-C. In etioplasts, the PSI-C protein was found only in the stroma and was rapidly turned over. In chloroplasts, the PSI-C protein was transiently present in the stroma. Integration into the PS I complex in the thylakoid membranes prevented the turn-over that took place with the stromal form of the PSI-C protein. The barley mutant *viridis-zb*^{63} is specifically deficient in PS I but the mutation does not alter the expression of the PS I genes. Some of the PS I subunits could be detected at levels of 1-5% of wild-type levels but no PS I activity could be detected. In the mutant, the PSI-D polypeptide accumulated in the stroma whereas only very small amounts were incorporated into the thylakoids.